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KAINATE-INDUCED NETWORK ACTIVITY IN THE ANTERIOR CINGULATE CORTEX

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Abstract—Anterior cingulate cortex (ACC) plays a pivotal role in higher order processing of cognition, attention and emotion. The network oscillation is considered an essential means for integration of these CNS functions. The oscillation power and coherence among related areas are often dis-regulated in several psychiatric and pathological conditions with a hemispheric asymmetric manner. Here we describe the network-based activity of field potentials recorded from the superficial layer of the mouse ACC *in vitro* using submerged type recordings. A short activation by kainic acid administration to the preparation induced populational activities ranging over several frequency bands including theta (3–8 Hz), alpha (8–12 Hz), beta (13–30 Hz), low gamma (30–50 Hz) and high gamma (50–80 Hz). These responses were repeatable and totally abolished by tetrodotoxin, and greatly diminished by inhibitors of ionotropic and metabotropic glutamate receptors, $GABA_A$ receptor or gap-junctions. These observations suggest that the kainate-induced network activity can be a useful model of the network oscillation in the ACC circuit.

Key words: anterior cingulate cortex, oscillation, kainate.

INTRODUCTION

The anterior cingulate cortex (ACC) receives sensory signals from somatosensory cortices and other areas, such as medial thalamus in the mouse (Yang et al., 2006) for nociceptive information. On top of simple pain sensation, it plays the integrative roles in the processing of cognition, attention and emotion. The cognitive function, particularly the top-down regulation of sensation encompasses multiple dimensions, such as, pain sensation of emotional or affective aspects (Rainville et al., 1997), placebo effects (Wager et al., 2004) and anxiety from observational fear in humans (Morrison and Downing, 2007). In particular, activation of ACC by observation of other's painful treatment attracts interests because it may suggest the ACC' role in ''empathy" in humans (Singer et al., 2004) or even in mice (Jeon et al., 2010). ACC is also activated in the conflict paradigm in human experiments (Takahashi et al., 2009), and suggested to be one of the most vulnerable brain areas to stressful insults. Actually in our previous work, chronic restraint stress load to mice induced disinhibition in the ACC circuit and increased synaptic plasticity of the excitatory synapse in the superficial layer as well as hyperlocomotion observed behaviorally (Ito et al., 2010).

The medial prefrontal cortex (mPFC) which includes ACC is responsible to sensory-motor gating, exemplified by pre-pulse inhibition (Swerdlow et al., 2001). Pre-pulse inhibition (PPI) is considered an endophenotype of schizophrenia. Reduced PPI for event-related potentials (ERPs) in front-central and front-temporal sites of scalp recordings is correlated with symptoms in schizophrenia patients (Bender et al., 1999). And also in rodent animal models of schizophrenia, reduction was reported in PPI of auditory startle responses, which was mediated by the prefrontal cortex (McNally et al., 2011) or limbic regions including the amygdala, and the dorsal hippocampus (Bakshi and Geyer, 1998).

Furthermore, in these pathological situations many studies report abnormalities in the network oscillation in ACC. The network oscillation is considered as the fundamental property emerged from the oscillators embedded in the neuronal network and also as an essential means for execution of the integrative brain functions such as cognition, decision-making and motor execution (Buzsaki and Draguhn, 2004; Schnitzler and Gross, 2005). In particular, these oscillation activities are observed in humans and animals during the tasks engaged in memory, attention and consciousness. Gamma band oscillation (GO, 30–70 Hz) is postulated to bind different visual features into a unified perceptual category (for review, Koenig et al., 1996). Visually evoked coincident activities were observed among widely separated regions of the human brain (Melloni et al., 2007). Enhancement of GO is observed in selective attention and short-term memory. Visual short-term memory, motor task anticipation and performance are also related with the beta band oscillation in the subthalamic nucleus (Kuhn et al., 2004) and the extra-striate visual cortex

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Abbreviations: ACC, anterior cingulate cortex; GO, gamma band oscillation; KA, kainate; mPFC, medial prefrontal cortex; PPI, pre-pulse inhibition; PSD, power-spectrum density; TTX, tetrodotoxin.

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(Tallon-Baudry et al., 2001) in humans. The hippocampal theta band oscillation is considered crucial to explorative activities and memory recall (for review, Buzsaki, 2002). Delta and/or slower oscillations in electroencephalogram (EEG) or in local field potentials are mainly observed in sleeping phases (Steriade et al., 1993) and are likely relevant to memory consolidation during sleep (Genzel et al., 2014).

Although molecular and cellular mechanisms of the network oscillation in the hippocampus and thalamo-neocortical circuits have been extensively investigated (Buhl et al., 1998; Fisahn et al., 1998; Buzsaki, 2002; Buzsaki and Draguhn, 2004; Roopun et al., 2006; Anderson et al., 2010; Ainsworth et al., 2011; Haggerty et al., 2013), detailed properties of the oscillation in the ACC-mPFC circuitry which has fundamental significance in pathophysiology in the psychiatric disorders are still largely unexplored. Therefore in the present study, we explored an *in vitro* model for the mouse ACC network oscillation to elucidate pharmacological profile of the kainate-induced network activity, particularly its involvement of excitatory and inhibitory transmitter receptors and gap-junctional communication.

EXPERIMENTAL PROCEDURES

Animal experimentation

All experiments were conducted in accordance with the experimental protocol approved by the Institutional Animal Care and Use Committee at Saitama Medical University (approval ID #: 1382).

Electrophysiological experiments

Electrophysiological experiments were carried out on brain slices from 74 of 5–6 week-old C57/BL mice. Mice were deeply anesthetized with isoflurane inhalation and then sacrificed by decapitation. The brain was quickly removed, and 450-μm coronal slices containing the ACC of both hemispheres in one section were rapidly prepared using a tissue slicer (Leica VT1000S, Germany). For slicing, a cutting solution was used in the following composition (in mM): 120 choline chloride, 3 KCl, 8 $MgCl_2$, 28 $NaHCO_3$, 1.25 NaH_2PO_4, 22 glucose.

Slices were placed in an interface holding chamber filled with a humidified mixed gas of 95% O_2–5% CO_2 to recover from tissue injuries for at least 1 h before recording. For recording, slices were transferred to a submerged-type recording chamber, and perfused at a rate of 6 ml/min with artificial cerebrospinal fluid (ACSF, in mM: 120 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 15 glucose) equilibrated with 95% O_2-5% CO_2 at 32–33 °C maintained by thermocontroller (WARNER Instrument Corporation, TC-324B, USA). We adopted the use of a submerged type chamber for the recordings because of its superior exchange rate of perfusion compared with interface-type chamber. Previous literatures suggested the fast perfusion rate (6 ml/min) can overcome poor oxygen condition inside of the slice tissue, which is inherent to the use of submerged type chamber (Hajos and Mody, 2009; Hajos et al., 2009; Lu et al., 2012).

Extracellular field potential recordings were performed from the superficial layers of ACC (Cg1 area) using a glass electrode pulled from borosilicate capillary (WPI, 1B150F-4, USA), and filled with 0.5 M NaCl. The tip resistance was between 2 to 4 Mohms with the internal recording solution (0.5 M NaCl). Signals were amplified by differential amplifier (WPI, DAM80, USA) and fed to a PC after filtering between 0.1 Hz and 1 kHz for offline analysis via an interface (Axon Instruments, Digidata 1440A, USA).

Although in the previous studies the deep (infragranular) layer is postulated to generate spontaneous or input-driven slow network oscillations in the sensory cortices (Sanchez-Vives and McCormick, 2000; Bon-Jego and Yuste, 2007; Sakata and Harris, 2009; Ainsworth et al., 2011; Beltaramo et al., 2013), we focused the superficial layer because much stronger network activities were observed in this layer compared with in the deep layer in the present study in the ACC (Fig. 1).

This kainate (KA)-induced network activity was considered to originate within the ACC circuit. First, it was observed in the recording from a 'microslice' containing only ACC–Cg1 area (data not shown). Next, the focal inactivation of neural excitation by tetrodotoxin (TTX, 100 μM in 0.5 M NaCl) diffused from the recording electrode greatly reduced the activity (data not shown).

Pharmacological treatment

In order to induce network activity, kainic acid (KA, 50 μM) was administered to the preparation by perfusion for 10 s in a submerge-type chamber, which allows a much rapider rise-and-fall as well as more uniform distribution of the drug concentration compared with the application using interface chambers. Our estimation of drug concentrations in the chamber using a dye solution (Phenol Red) indicates that the perfusate was diluted ca. 1 to the fourth from the original solution (*i.e.*, ca. 12–13 μM) at a peak time of 20 s after dye application and that the concentration rapidly declined to 1/30 at 60 s.

Kainate-induced network activity was evoked 4 times in each slice with 5–10-min intervals, unless otherwise stated. Pharmacological examinations were done on the 3rd and 4th trials.

The role of the voltage-sensitive sodium channel in the KA-induced activity was examined by perfusion of tetrodotoxin (TTX, 1 μM) starting at 10 min prior to the 3rd KA stimulation until the end of the 4th KA stimulation. Contribution of the glutamate receptors were estimated by application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) for the AMPA/KA receptor; D (-) -2-amino-5-phosphono-pentanoic acid (D-APV, 50 μM) for the NMDA receptor; and a blocker of metabotropic glutamate receptor, (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA, 100 μM) for group 1 metabotropic glutamate receptors with the same time schedule as TTX.

Involvement of $GABA_A$ receptor-mediated transmission in the network activity was investigated by focal application of bicuculline (500 μM in 0.5 M NaCl),